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14. ABSTRACT Neurofibromatosis type 2 (NF2) is an autosomal dominant disorder, characterized by the development of bilateral vestibular and spinal schwamomas, meningiomas, and ependymomas. The hF2 gene encodes a 595 amino acid polypeptide known as NF2 protein or Merlin or Schwannomin. The primary structure of the NF2 protein is homologous to the ERM family of peripheral membrane proteins, which includes Ezrin, Radixin, and Moesin. The founding member of the ERM superfamily is the erythrocyte membrane protein 4.1, which cross-link's spectrin-actin complexes and attaches them to the plasma membrane. We have established that p55, a palmitoylated peripheral membrane phosphoprotein, forms a ternary complex with protein 4.1 and glycophorin C. Notably, the Drosophila homologue of p55 functions as a tumor suppressor in epithelial and neuronal tissues. In the 2nd year of the funding period, we demonstrated binding between p55 and the NF2 protein and established the existence of this complex in human erythrocyte plasma membrane. This unexpected finding revealed a new paradigm, integrating the known functions of the p55 family of proteins with the pathophysiology of the NF2 protein.					
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Progress Report (Year 3)

Career Development Award # NF020087

PI: A.H. Chishti

Title: Biochemical characterization of native Schwannomin/Merlin

OBJECTIVE

As outlined in the Statement of Work (SOW), Task # 3 proposed a search for Schwannomin-binding protein(s) homologue in neuronal cells and establishes the physiological basis of their interactions in the nervous system.

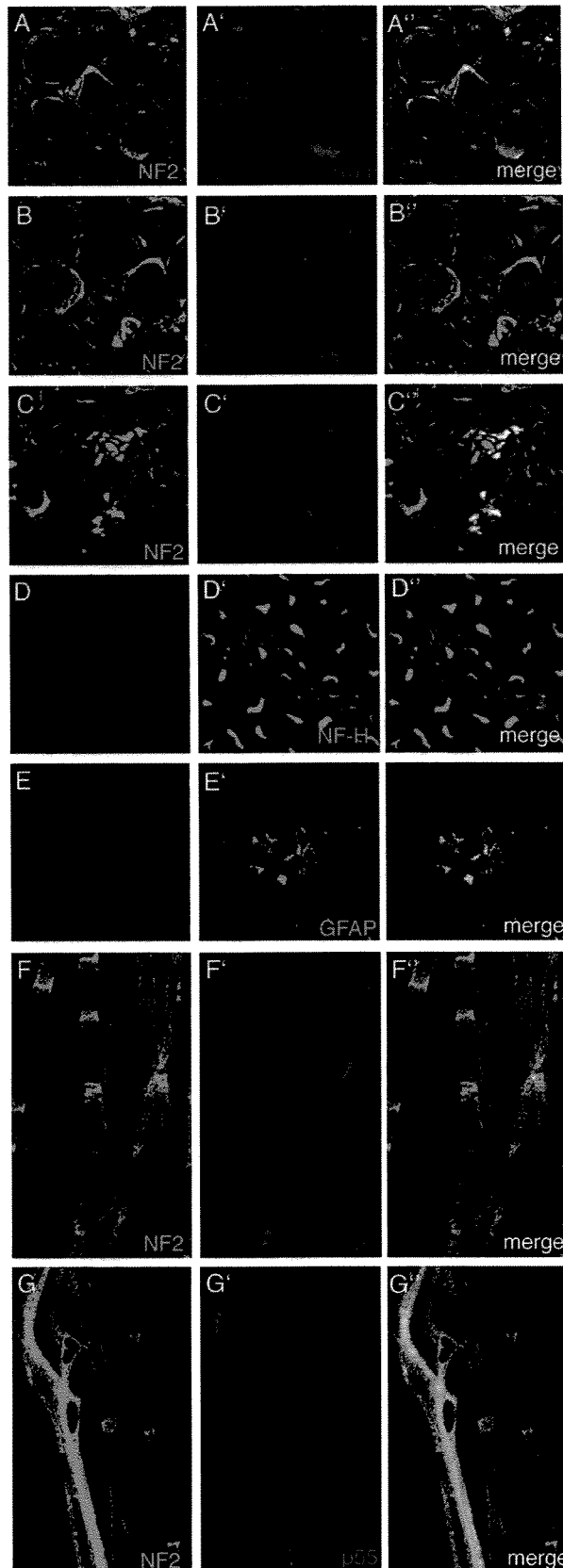
SUMMARY OF THE WORK COMPLETED

Neurofibromatosis type 2 (NF2) is an autosomal dominant disorder, characterized by the development of bilateral vestibular and spinal schwannomas, meningiomas, and ependymomas. The NF2 gene encodes a 595 amino acid polypeptide known as NF2 protein or Merlin or Schwannomin. The primary structure of the NF2 protein is homologous to the ERM family of peripheral membrane proteins, which includes Ezrin, Radixin, and Moesin. The founding member of the ERM superfamily is the erythrocyte membrane protein 4.1, which cross-link's spectrin-actin complexes and attaches them to the plasma membrane. We have established that p55, a palmitoylated peripheral membrane phosphoprotein, forms a ternary complex with protein 4.1 and glycophorin C. Notably, the *Drosophila* homologue of p55 functions as a tumor suppressor in epithelial and neuronal tissues. In the 2nd year of the funding period, we demonstrated binding between p55 and the NF2 protein and established the existence of this complex in human erythrocyte plasma membrane. This unexpected finding revealed a new paradigm, integrating the known functions of the p55 family of proteins with the pathophysiology of the NF2 protein.

In the 3rd year, we organized all the data for the purification of the NF2 protein from human erythrocyte membrane and assembled a detailed manuscript for publication. After several revisions and additional experimentation, the manuscript was recently accepted for publication. The details are as follows: Jindal, H.K., Yoshinaga, K., Seo, P.S., Lutchman, M., Dion, P.A., Rouleau, G.A., Hanada, T., and Chishti, A.H. (2006). Purification of the NF2 tumor suppressor protein from human erythrocytes. *The Canadian Journal of Neurological Sciences (in press)*. This manuscript did not include our recent findings showing the binding of the FERM domain of NF2 with p55. To extend these findings, a critical requirement was to demonstrate the presence of p55 in neuronal cells where the NF2 protein is abundantly expressed. Since the existing polyclonal antibodies do not generally recognize non-erythroid p55 antigens, we set out to generate a monoclonal antibody that can detect the p55 antigen in all tissues. Using this newly developed monoclonal antibody, we demonstrated the presence of p55 in non-myelin forming Schwann cells. Importantly, the co-localization studies showed a perfect co-localization of p55 and NF2 proteins in Schwann cells. These results suggest that the biochemical interaction between p55 and NF2 protein might play a physiological role in

Schwann cells. Currently, we are investigating the physiological significance of this work by using the p55 null mouse model recently developed in our laboratory.

EXPERIMENTAL PROCEDURES AND RESULTS



Development of new monoclonal antibody against human erythrocyte p55. Recombinant GST fusion protein containing the SH3 and GUK domain construct of human erythrocyte p55 was injected into mice. The immune response was monitored using a His-tagged fusion of SH3-GUK domain construct. The 2G4 monoclonal works fine in immunoprecipitation and immunocytochemistry applications. The isotype of this monoclonal is IgG2b. The epitope of this antibody is located within the guanylate kinase-like (GUK) domain of p55.

Immunohistochemistry of transverse section of rat sciatic nerves.

Samples of rat sciatic nerve transverse section were stained with anti-p55 monoclonal antibody, anti-NF2 polyclonal (A-19, Santa Cruz Biotechnology), along with markers for both myelin forming and non-myelin forming Schwann cells (S100), for axon (NF-M), and for non-myelin forming Schwann cells (GFAP). The NF2 protein co-localized with S100 and GFAP, but not with NF-M (Fig. 1, A, B, C), confirming its expression in Schwann cells. P55 did not co-localize with NF-M (Fig. 1, D), and co-localized with GFAP (Fig. 1, E). These results suggest that p55 is preferentially expressed in non-myelin forming Schwann cells in rat sciatic nerve, and not in axons.

Immunohistochemistry of mouse nerves teased fibers. NF2 was stained along with Caspr, which is the marker for paranode, in teased fibers of mouse nerves. Prominent staining of NF2 was observed at Schmidt-Lanterman incisures as well as paranode (Fig. 1, F) as previously reported. Double staining of p55 and NF2 shows extensive co-localization of two proteins in non-myelin forming Schwann cells (Fig. 1, G). In myelin-forming Schwann cells, the expression of p55 was not detected. In conclusion, p55 and NF2 co-localize in non-myelinated fibers in mouse nerves.

CONCLUSIONS

- (1) We have developed a specific monoclonal antibody against human erythrocyte p55. This antibody can also detect p55 from mouse and rat tissues, and works fine in both Western blotting and immunocytochemistry applications.
- (2) The development of the p55 monoclonal antibody permitted us to demonstrate the preferential expression of p55 in non-myelin forming Schwann cells in rat sciatic nerves, but not in axons.
- (3) The p55 and NF2 proteins co-localize in non-myelinated fibers in mouse nerves but not in myelin-forming Schwann cells.
- (4) We have also generated a p55 knockout mouse model. Currently, we are investigating whether the membrane association and other biochemical properties of the NF2 protein are altered in the absence of p55 in erythrocytes and Schwann cells.

TRAINING ACCOMPLISHMENTS

Financial support provided under the Career Development Award enabled Dr. Chishti to utilize his extensive experience of erythrocyte membrane biochemistry in discovering new biochemical properties of the NF2 protein. This focus finally led to the discovery of p55 as the binding partner for the NF2 protein. The p55 protein is a major focus of Dr. Chishti's research interests. All necessary reagents are in place, and the p55 knockout mouse phenotype is currently under investigation. The co-localization of p55 and NF2 proteins in Schwann cells further underscores the physiological relevance of these findings for understanding the pathogenesis of neurofibromatosis type 2. The resources generated during the course of these studies will be invaluable for the future characterization of p55-NF2 protein interactions by Dr. Chishti and other laboratories. Funding under this program enabled Dr. Pil Seo to learn valuable biochemical techniques required for the characterization of p55-NF2 protein interactions and Dr. Toshi Hanada, a Research Assistant Professor, played a key role in the development of p55 monoclonal and its subsequent characterization in Schwann cells.